1. THE INVENTION

The present invention relates to recombinant adeno-associated virus vectors for gene delivery and regulated tissue specific expression in a host. The vectors of the invention contain a mammalian gene of interest, cis-acting regulatory and promoter elements of the gene of interest and an adeno-associated virus vector engineered in such a way that the expression of the gene is regulated in a tissue specific manner by the cis-acting regulatory and promoter elements.

The vectors of the invention may be used for therapeutic purposes. More specifically, the vectors may be used to deliver and express, in a tissue specific manner, a mammalian gene for therapeutic purposes. The recombinant adeno-associated virus vectors may be used to treat a variety of different genetic or acquired diseases including Sickle cell anemia, β-thalassemia, Gaucher's disease, Parkinson's disease and Cystic Fibrosis.

2. THE REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH, SHOULD BE WITHDRAWN

Claims 1-35 and 39 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or which it is most nearly connected, to make and/or use the invention.

According to the Examiner, Applicants have not taught how to use a recombinant adeno-associated virus vector for a therapeutic purpose. More specifically, Applicants have not taught how to achieve successful expression of an introduced

gene in cells *in vivo*, such that the transduced cells express the gene to a sufficient level, that enough cells are transduced and successfully taken up by the patient, and that the introduced gene is stably maintained in the target cells and transferred to progeny cells so as to achieve a desired therapeutic effect.

The test for enablement is whether one reasonably skilled in the art could make or use the invention, without undue experimentation from the disclosure in the patent coupled with information known in the art at the time the patent was filed. *U.S. v. Telectronics*, Inc., 857 F.2d 778, 8 USPQ2d 1217 (Fed.Cir. 1988).

The instant specification, as filed, describes (i) methods for recombinantly engineering adeno-associated virus vectors containing a mammalian gene of interest and the *cis*-acting regulatory and promoter elements of that gene, (ii) methods for transducing the recombinant adeno-associated virus into mammalian cells, and (iii) methods for detecting the expression of the desired gene in the transduced cells.

For example, the specification discloses methods for recombinantly engineering adeno-associated vectors containing the human gamma globin gene linked to *cis*-acting regulatory elements (see page 46, lines 8-20 of the specification). The specification describes methods for purification of human bone marrow progenitors from a patient host (see page 38, lines 7-15 of the specification). Further, the specification teaches transduction of mammalian cells, such as Detroit 6, erythroleukemia cells and hematopoietic cells using the

recombinantly engineered adeno-associated virus vectors of the invention (see page 38, lines 22-30 of the specification).

Applicants maintain that given the specific teachings of the specification, one skilled in the art could, without undue experimentation, transduce mammalian cells with recombinant adeno-associated virus vectors to affect a therapeutic benefit for treatment of mammalian diseases or disorders. All that is required is that the skilled artisan follow the teachings of the specification.

Further, as exemplified in the working examples of the specification, recombinantly engineered adeno-associated viral vectors were successfully used to introduce and achieve regulated expression of globin gene expression in an erythroid derived cell line. As indicated on page 26, lines 24-28 of the specification:

> "High level, regulated globin expression was obtained and efficient integration into the genome without rearrangement occurred in all clones studied. Moreover, the messenger RNA expression of the transduced gene was comparable to endogenous gamma globin levels. The correct globin start site was utilized in the transduced gene and tissue specific expression which was hemin inducible was maintained."

Additionally, the Examiner's attention is invited to the Rule 132 Declaration of Dr. Richard Jude Samulski (the "Samulski Declaration"), submitted herewith as Exhibit A. As described in the Samulski Declaration, experiments were conducted in which primate bone marrow progenitor cells were isolated, transduced using recombinant adeno-associated virus vectors, and transferred back into a γ-irradiated primate host. As indicated by the data presented in the Samulski Declaration, the transferred viral transgene could be detected

in the peripheral blood mononuclear cells (PB) and bone marrow (BM) from three of the six experimental animals (See ¶6, Samulski Declaration). Further, in one experimental animal the transgene could be detected for up to three months following transduction. Colony forming unit (CFU) assays indicated that the transduction of recombinant AAV into bone progenitor cells did not adversely affect reconstitution, and as shown, both myeloid and lymphoid lineages contained the transferred transgene (See ¶7, Samulski Declaration). Additionally, the ability of transduced cells to grow in the presence of G418 indicates successful expression of a functional protein (See ¶8, Samulski Declaration).

Thus, Applicants have demonstrated (i) the successful adeno-associated virus mediated transfer and tissue specific expression of a globin transgene in erythroid cells, (ii) the successful *in vivo* transfer of AAV DNA into bone marrow progenitor cells for up to three months following transduction, (iii) that AAV transduction of bone marrow progenitor cells does not adversely affect reconstitution of the transplanted animals, and (iv) that the transduced cells are capable of expressing a functional protein.

Applicants assert that the instant specification is fully enabled for the pending claims. If the Examiner has reason to doubt the teaching of the specification as it relates to the specific subject matter of the pending claims, applicants respectfully request that the Examiner provide a citation directed to references that support such a position or an affidavit as provided for by 37 C.F.R. § 1.107.

For all the aforementioned reasons, Applicant respectfully submits that the §112, first paragraph, enablement rejections be withdrawn.

CONCLUSION

Entry of the foregoing remarks into the file of the above-identified application is respectfully requested. Applicants believe that the invention defined by the claims meets all the requirements for patentability. Withdrawal of all rejections and reconsideration of the amended claims so requested. An early allowance is earnestly sought.

Respectfully submitted,

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Enclosure